component, a minor constituent, travels relatively fast and concentrates at the front, as is shown in Fig. 1. During the first 6 hr of resolution, the descending chromatogram exhibits this minor component, but it is lost through diffusion on further development. After 48 hr, the descending chromatogram did exhibit two components but in a decidedly diffused and streaked condition.

The simplicity and rapidity of the new technique affords the laboratory a continuous system for ascending chromatography at a minimum cost.

References

1. R. J. Williams and H. Kirby, *Science* **107**, 481 (1948).
2. J. C. Underwood and L. B. Rockland, *Anal. Chem.* **26**, 1553 (1954).

15 January 1955.

Detoxication of Drugs and Other Foreign Compounds by Liver Microsomes

Bernard B. Brodie, Julius Axelrod, Jack R. Cooper, Leo Gaudette, Bert N. La Du, Choco Mitoma, Sidney Udenfriend

Laboratory of Chemical Pharmacology, National Heart Institute, National Institutes of Health, Bethesda, Maryland

Recent studies demonstrating that diethylaminoethyl diphenylpropylacetate HCl (SKF 525-A) inhibits the rate of biotransformation of drugs metabolized by a diversity of metabolic pathways (*1, 2*) suggested that the tissue catalysts responsible for their metabolism possess certain factors in common. This report describes experiments which show that common denominators in the metabolism of a variety of foreign compounds (Table 1) are most unusual and include localization of the enzyme systems in liver microsomes and requirements for both reduced triphosphopyridine nucleotide (TPNH) and oxygen.

Table 2. Requirements for the demethylation of monomethyl-4-aminoantipyrine by dialyzed rabbit liver homogenate.

Additions	Amount of 4-aminoantipyrine formed (μM)
Complete system*	1.53
Minus Mg^{++}	0.99
Minus glucose-6-phosphate	0.87
Minus nicotinamide	0.15
Minus TPN	0.14
Diphosphopyridine nucleotide in place of TPN	0.20

* To 2 ml of liver homogenate (1 : 2 in 0.2*M* phosphate buffer, *p*H 7.4) were added 100 μM of nicotinamide, 75 μM of $MgCl_2$, 0.2 μM of TPN, and 5 μM of monomethyl-4-aminoantipyrine to a final volume of 5 ml. Incubation was for 1 hr at 37°C in air.

From the results in Table 2, which show the requirements for the demethylation of monomethyl-4-aminoantipyrine in homogenates of dialyzed rabbit liver, it is seen that the reaction is enhanced by nicotinamide, triphosphopyridine nucleotide (TPN), Mg^{++}, and glucose-6-phosphate. Similar requirements were also found for the metabolism of the other drugs listed in Table 1.

The cellular localization of the biochemical reactions was determined by measuring the activity of cellular fractions separated by differential centrifugation of liver homogenates (*11*). Table 3 shows that both microsomal and soluble fractions of liver are required for drug metabolism. Nuclei and mitochondria, on the other hand, show virtually no activity.

The role of the soluble fraction in the reactions was suggested by the afore-mentioned stimulation by glucose-6-phosphate together with the presence of considerable glucose-6-phosphate dehydrogenase activity in the soluble fraction. These observations indicate that TPN might be required in the homogenate in the reduced form, TPNH, and that glucose-6-phosphate

Table 1. Reactions catalyzed by enzyme systems in liver microsomes. Analytic methods are described in the references cited.

Type of reaction	Substrate	Products	Reference
Barbiturate side-chain oxidation	Evipal (hexobarbital)	Keto-evipal	(*3*)
	Nembutal (pentobarbital)	Nembutal alcohol and nembutal carboxylic acid	(*4*)
Dealkylation	Pyramidon (dimethyl-4-aminoantipyrine)	4-Aminoantipyrine + formaldehyde	(*5*)
	Monomethyl-4-aminoantipyrine	4-Aminoantipyrine + formaldehyde	(*5*)
	Monoethylaniline	Aniline + acetaldehyde	(*6*)
	Ephedrine	Norephedrine + formaldehyde	(*7*)
Deamination	Benzedrine (amphetamine)	Phenylacetone + ammonia	(*8*)
Ether cleavage	Codeine	Morphine + formaldehyde	(*9*)
	Phenacetin (*p*-ethoxyacetanilide)	*p*-Hydroxyacetanilide + acetaldehyde	(*9*)
Hydroxylation	Aniline	*p*-Hydroxyaniline	(*10*)
	Acetanilide	*p*-Hydroxyacetanilide	(*10*)

dehydrogenase or some other TPNH-producing enzyme is an important component of the soluble fraction. Substantiation of this hypothesis was afforded by the finding that the substrates in Table 1 are metabolized in washed microsomes incubated with TPN, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase at rates comparable to those in the whole homogenate. The addition of chemically prepared TPNH to washed microsomes also effects the metabolism of the various drugs—direct evidence that TPNH is involved in the various reactions. It may be concluded from these results that the drug enzyme systems are located in the microsomes and that the soluble fraction participates by maintaining TPN in the reduced form.

A number of observations indicate that the metabolic pathways in Table 1 are not catalyzed by a single enzyme system. For example, microsomes prepared at *p*H 7.0 rapidly lose their ability to hydroxylate aniline and acetanilide but not to dealkylate monomethyl-4-aminoantipyridine; SKF 525-A inhibits most of the reactions in Table 1 but does not appreciably affect the hydroxylation of aniline and acetanilide; the inhibitor blocks the ether cleavage of phenacetin but not of codeine.

It is unusual for enzyme systems to require both TPNH and oxygen. A common step in the various microsomal reactions could involve the production of hydrogen peroxide by the oxidation of TPNH. The generated peroxide might then be utilized by peroxidaselike enzymes to catalyze the transformation of the various foreign compounds. It is likely that the number of these enzymes is relatively small and that they are unusually nonspecific.

The distribution of the drug enzyme systems in various rabbit tissues was examined and, in general, the various drugs were found to be metabolized only by the microsomes in liver. The localization of these functions in liver is of particular interest since it imparts an unusual specialization to the submicroscopic particles of this tissue.

Table 3. Cellular localization of enzyme systems catalyzing hydroxylation of acetanilide and demethylation of monomethyl-4-aminoantipyrine in rabbit liver.*

	Percentage of total activity†	
	Hydroxylation	Demethylation
Whole homogenate	100	100
Microsomes	1	3
Soluble fraction	0	3
Microsomes + soluable fraction	77	102

* *Incubation conditions.* Hydroxylation system : Flasks contained 1 ml of liver homogenate (1 : 2) in isotonic KCl, 5 μM of nicotinamide, 0.25 μM of TPN, 10 μM of acetanilide, and 0.5 ml of 0.5*M* tris-phosphate (1 : 1) buffer, *p*H 8.5 ; final volume, 3.5 ml. Incubation was for 1 hr at 37°C in air. Dealkylation system : As described in Table 2.

† Whole homogenate activity taken as 100 percent.

It is interesting to consider the question of whether or not the metabolism of foreign compounds by the microsomal enzyme systems is merely incidental to the normal regulation of body processes. In view of the relative nontoxicity of SKF 525-A, one can speculate that these systems are not essential to the normal economy of the body, but operate primarily against the toxic influences of foreign compounds that gain access to the body from the alimentary tract.

References

1. J. Axelrod, J. Reichenthal, B. B. Brodie, *J. Pharmacol. and Exptl. Therap.* **112**, 49 (1954).
2. J. R. Cooper, J. Axelrod, B. B. Brodie, *ibid.*, **112**, 55 (1954).
3. J. R. Cooper and B. B. Brodie, *ibid.* **110**, 12 (1954).
4. ———, in preparation.
5. B. N. La Du *et al.*, *J. Biol. Chem.*, in press.
6. B. N. La Du, L. Gaudette, B. B. Brodie, in preparation.
7. J. Axelrod, *Federation Proc.* **13**, 332 (1954).
8. ———, *J. Pharmacol. and Exptl. Therap.* **110**, 2 (1954).
9. ———, in preparation.
10. C. Mitoma and S. Udenfriend, *J. Pharmacol. and Exptl. Therap.*, in press.
11. W. C. Schneider and G. R. Hogeboom, *J. Biol. Chem.* **183**, 123 (1950).

4 January 1955.

Dicentrics in Wheat

J. W. Morrison

Cereal Crops Division, Central Experimental Farm, Ottawa, Canada

Dicentric chromosomes are chromosomes that have two centromeres. They are no longer oddities in biological research because they commonly occur after breakage and reunion in chromosomes following irradiation or a treatment with some radiomimetic substance. No observations of the behavior at meiosis of artificially produced dicentrics have been reported, because few of them have persisted. Usually they are altered or eliminated in the mitotic divisions and do not reach the germ line. There have been only a few naturally occurring dicentrics (*1*). Two naturally occurring dicentrics persisted in wheat and have been studied at both mitosis and meiosis (*2*). The behavior of dicentrics at meiosis is especially interesting because the centromere plays an important role in pairing, chiasma formation, and chromosome movement—phases of activity about which there is still very little known. This report concerns the progress of a 2-yr investigation into a possible source of dicentrics.

In a previous paper I outlined a theory for the formation of dicentric chromosomes. I proposed that the dicentrics arose after fracture of univalent chromosomes at meiosis with the fusion of two broken ends in a subsequent resting stage. This theory was based on observations of meiotic stages in parental pentaploid material and also, of course, on the occurrence of a dicentric chromosome in one plant of an F_2 progeny. To test this hypothesis further, the interspecific cross *Triticum aestivum* ($2n = 42$) × *T. durum*